

REMARKS

Applicants thank the Examiner for rejoining Group I and Group II claims.

Applicants submit a supplemental IDS, and respectfully request the Examiner to consider the references cited therein.

In a good faith attempt to place this application in condition for allowance, Applicants offer extensive narrowing and clarifying amendments to the presented claims, and have reduced their number.

Claims 1-42 were presented for examination and stand rejected. Claims 1-6, 13, 20, 23, 24, 28, and 30-34 have been amended. Claims 10-12, 14, 15, 17, 18, 22, 25, 29, and 35-41 have been canceled without prejudice. Dependent Claims 7-9, 16, 19-21, 26, and 27 remain unchanged. New Claims 42-47 have been added to explicitly cover a preferred embodiment of the invention. Support can be found throughout the specification, including the original claims. See, for example, the second to the last paragraph on page 42 for Claim 47.

All claims are believed to be novel and unobvious in view of the applied references, and indeed over all references known to Applicants, and are submitted to be in condition for allowance.

As will be clear from a review of the presented claims, all of which are clearly based in the specification, the underlying basis of the invention as now claimed is the ability to generate binders to relatively small, linear peptides (URs or PETs) that are chosen to be unique to the protein from which they are derived in the context of the sample, and reproducibly to be present and exposed on digestion fragments. Through this basic approach, the invention for the first time, as far as Applicants are aware, permits generation of reproducible and quantitative array-generated data, indicative of the presence, and at least relative concentrations of plural proteins in a biological sample. The results achieved using a multiplexed array of binders specific for unique restriction sequences (URS, sometimes referred to herein as Protein Epitope Tags, or PETs), as Applicants have taught permits reproducible multiplexed detection of plural proteins simultaneously. This permits the invention to be used in analyses wherein proteomic profiles are determined.

Applicants believe the era of diagnosis, prognosis, and biological state or species determination based on assay for the presence or quantity of a single or a few markers (characteristic biomolecules) will give way to the determination of a pattern of plural biomarker molecules. This invention addresses a convenient means and method of conducting such multiplexed analysis. Furthermore, Applicants' claimed methods which permit detection of protein isoforms, including splice variants (see new Claims 42-46), a feature Applicants believe to be extremely valuable, and neither disclosed nor suggested in the prior art.

Another important aspect of the invention as now claimed is the development of multiplexed sandwich assays in combination with the use of PETs, where antibody to one exposed epitope defined by a PET is immobilized in an array, and antibody to another different exposed epitope is used to complete the sandwich (see, for example, Claims 1 and 5 as amended).

With the above argument in mind, Applicants now turn to address the specific issues raised by the Examiner in the December 28, 2005 Office Action.

Claim objections

The Office Action objects to Claims 1, 4, 5, and 29 because of a few formality issues.

Due to the amendments to Claims 1, 4, and 5, and the cancellation of Claim 29, these objections are either overcome or rendered moot. Reconsideration and withdrawal of the objections are respectfully requested.

Claim rejections under 35 U.S.C. § 112, second paragraph

The Office Action rejects Claims 1-29 and 32-36 as being indefinite.

Due to the amendments to Claims 1, 3, 20, and 32-34, and the cancellation of Claims 35 and 36, these rejections are either overcome or rendered moot. Reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph are respectfully requested.

Claim rejections under 35 U.S.C. § 103

Claims 1-41 are rejected under 35 U.S.C. § 103(a) as unpatentable over Ault-Riche et al. (US 2003-0143612 A1) in view of Wagner et al. (U.S. Pat. No. 6,897,073 B2). Applicants respectfully traverse this rejection to the extent applied to the claims as amended. Applicants submit that, in view of the amendments to the claims and the arguments above, the cited references, either individually or in combination, clearly fail to disclose or suggest the subject matter of the claimed invention taken as a whole.

Merely to illustrate, for example, amended Claim 1 includes a sample fragmentation step (step (1)), which calls for *predetermined* sample *denaturation and proteolysis*. One reason for carefully selecting and pre-determining the denaturation and proteolysis processes is to ensure that the PETs to be detected in the claimed methods *remain intact* after the sample treatment. Protein digestion without taking into consideration the location of the PETs in the target proteins (*e.g.*, whether the PETs happen to encompass an internal protease recognition site) will likely be *incompatible* with the claimed invention. In addition, pre-determined denaturation condition allows standardization of proteolysis, contributing to *reproducible* PET exposure and more consistent protein digestions patterns.

In contrast, as recognized in the Office Action, Ault-Riche does not teach sample denaturation and cleavage (digestion).

However, the Office Action does assert that Wagner teaches, in column 35, lines 22-44, a method that uses “cleaved or denatured protein analytes (membrane bound proteins) from body fluids.” Applicants respectfully disagree.

The referred-to passage in Wagner at best teaches the use of “cellular extract,” or its digested products; the use of “solubilized” membrane proteins; and the use of “fragments of the expression products of a cell.” There is no teaching or suggestion in this passage that the protein should be denatured. In fact, Applicants have been unable to find in the Wagner specification a teaching or suggestion of the use of denaturation and proteolysis for sample preparation.

Another important difference relates to the origin of the epitope tags. The claimed methods use carefully selected, *endogenous* peptide sequences from the target proteins as PETs. In contrast, Ault-Riche teaches a completely different method, with a pre-requisite of

incorporating into a target protein certain *heterologous* epitope tags before the method can work (see, for example, paragraph [0011]). In fact, Ault-Riche devotes a whole subsection (paragraphs [0134] – [0157]) to discuss the various ways such *heterologous* epitope tags can be coupled to / incorporated into target proteins. Ault-Riche contains no teaching or suggestion that *endogenous* peptide fragments from the target proteins can be used as epitope tags for their detection or quantitation. Wagner is completely silent about the use of carefully selected PETs that remain intact in digested peptide fragments, despite the passing reference to the generic term “fragments.”

Furthermore, as argued above, neither reference appears to disclose the use of secondary capture agents in multiplexed sandwich assays (see amended Claim 1 and new Claim 42), and neither discloses or suggests, e.g., the detection / quantitation of *splice variant proteins* (see Claims 30 and 42).

Therefore, the applied references, either alone or in combination, still fail to teach or suggest the claimed invention. At least one of the three requirements of making out a *prima facie* case of obviousness under 35 U.S.C. § 103(a) is not met. Reconsideration and withdrawal of the obviousness rejection are respectfully requested.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims are now in condition for allowance and early notification to this effect is earnestly solicited. Any questions arising from this submission may be directed to the undersigned at (617) 951-7000.

No fee is believed to be due. If there are fees due in connection with the filing of this submission, please charge the fees to our **Deposit Account No. 18-1945**, under **EPTM-P03-001**. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our **Deposit Account No. 18-1945**.

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Respectfully submitted,

By


Yu Lu, Ph.D.

Registration No.: 50,306
Fish & Neave IP Group
ROPES & GRAY LLP
One International Place
Boston, Massachusetts 02110-2624
(617) 951-7000
(617) 951-7050 (Fax)